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Comparative study on the valorization of *Sargassum* from the Mexican Caribbean coast and Gulf of California as an ingredient on healthy diets for shrimp farming

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ABSTRACT

Sargassum biomass is a potential ingredient for aquaculture formulations due to its high nutritional value and availability, as it contains a variety of essential nutrients including proteins, carbohydrates, lipids, vitamins, antioxidants, fiber, and minerals, which are essential for aquatic growing, development and health parameters including digestibility and immune response against diseases. Therefore, in this work, five experimental diets were formulated considering 2.5 % and 5 % levels of inclusion of *Sargassum* spp. from the Mexican Caribbean coasts (SC), and from the Gulf of California (SG), and one reference diet as a control. The characterization of the raw material and the proximal analysis of the five diets were evaluated according to standard reference methods. A 35-day feeding trial was conducted using juvenile *Litopenaeus vannamei* showed a shrimp survival rate of 97 ± 6 % ($p > 0.05$) on SC. Excellent weight gain (WG) performance of SC and SG diets at 2.5 % of inclusion resulted in >10 % WG compared to the control. The specific activity of amylase, lipase, trypsin, and chymotrypsin showed a significant increase by including *Sargassum* in diets, compared with the reference diet. Incorporating *Sargassum* at 2.5 % in feed formulations for shrimps showed a great opportunity to use it as an ingredient and improved production yields. The digestion and absorption parameters were represented by the enzymatic activity in which the diets based on *Sargassum* spp. from the Mexican Caribbean coast (SC) have resulted in the best performance.

1. Introduction

Shrimp aquaculture is an economic activity that has shown rapid growth in recent decades worldwide, consuming around 70 % of the shrimp produced by the aquaculture industry (Olmos Soto, 2021). Between 2002 and 2027, the aquaculture industry is expected to grow at a rate greater than 7.1 %. While by 2050, food consumption from aquaculture will be 60–100 %, as the world population is projected to increase from 7.6 to 9.8 billion (Ahmad et al., 2022). The importance of shrimp farms lies in the fact that they are a rich source of nutrients, such as essential amino acids, vitamins, and minerals, and have high levels of polyunsaturated fatty acids (PUFA), which are necessary for human health (Panini et al., 2017).

On the other hand, one of the significant limitations that affect shrimp aquaculture profitability is the strict feeding of the shrimp. The shrimp diet is based on fishmeal and fish oil, which promotes optimal shrimp growth, making its production more expensive, and raising its costs in the market (Lee et al., 2020; Olmos Soto, 2021). As alternatives for fish-based ingredients, the development of feed formulations using economic vegetables has been used, but the major disadvantage is that

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the shrimp microbiota is unable to digest the complex macromolecules (lipids, carbohydrates, and proteins) from vegetable sources (Lee et al., 2020; Olmos Soto, 2021). Additional disadvantages of these vegetable-based feeds are that they create an ideal environment for the growth of opportunistic pathogens given that the shrimp does not consume all the components in the formulation, affecting the health and causing the death of the shrimp (Lee et al., 2020; Olmos Soto, 2021).

Therefore, the shrimp aquaculture industry seeks the implementation of new feed formulations that are economical, sustainable, healthy, and provide high nutritional value.

In this sense, *Sargassum* is a brown macroalgae genus in tropical and subtropical environments worldwide (Amador-Castro et al., 2021; Devault et al., 2021). It also has a high nutritional value, being a rich source of minerals, vitamins, proteins, and carotenoids. In addition, the obtention of diverse bioactive compounds such as tannins, flavonoids, glycose, sulfated polyphenols, phenolic, polysaccharides, and B-carotene, among others, from *Sargassum* have been reported. These metabolites have antimicrobial, anti-inflammatory, antioxidant, hepatoprotective, anti-tumor, and analgesic properties (Abdel-Rahim et al., 2021a; Rushdi et al., 2020). The world production of seaweeds in 2018 by farming methods and wild capture reached 34.4 million tons, representing an economic volume of USD 13.3 billion (Cai et al., 2021).

Currently, most of the studies reported in the literature use *Sargassum* spp. or the compounds derived from it in shrimp aquaculture to exert resistance against bacteria (vibriosis) or viruses (White Spot Syndrome Virus) by stimulating the expression of genes related to the immune system (Ghosh, Panda, and Luyten, 2021; Lee et al., 2020; Yudiati et al., 2019). Few studies report its implementation in shrimp diets to enhance growth performance or nutrient intake (Abdel-Rahim et al., 2021b; Lee et al., 2020), as is the case of Abdel-Rahim et al. (Abdel-Rahim et al., 2021b; Lee et al., 2020), who designed a formulation based on *Sargassum polycystum* and nucleotides to feed juvenile *Litopenaeus vannamei* for 56 days. The results indicated an increase in shrimp growth, immune response improvement, and cold tolerance. The most innovative strategy for shrimp feeding is the use of different natural and renewable compounds as additives that enhance the immune response to disease and stress resistance. Also, immune system stimulation is associated with increased growth. Different compounds present in *Sargassum*, such as polysaccharides, have been described as having pharmacological properties such as antioxidants, immunomodulators, hematopoiesis, and gastrointestinal protection, which are alternatives to reduce or replace the use of synthetic compounds such as levamisole and antibiotics, widely used to promote the immunomodulatory system and prevent bacterial diseases (Abbas et al., 2023).

More research needs to be conducted regarding the utilization of *Sargassum* spp. gathered from the Mexican coasts to develop aquafeed formulations. This renewable resource has been readily accessible since 2011, when the Mexican coasts suffered an unusual and extensive invasion of Sargassum. This massive increase of *Sargassum* is probably caused by climate change, meteorological phenomena, such as the lack of cyclones (which prevents the dispersal of *Sargassum*), and the increase of nutrients in the Mexican coasts that come from the Amazon River, the Mississippi River, and the Sahara Desert (Louime et al., 2017). As for the Amazon River, it carries a large amount of nutrients from the deforestation of the Amazon jungle. While the Mississippi River transports nutrients (nitrogen and phosphate) from neighboring farms (Lapointe et al., 2014; Turner and Rabalais, 1994; White et al., 2014). Concerning the sand of the Sahara, the oceans ingest this sand rich in nutrients such as iron, phosphate, and potassium (Louime et al., 2017). This massive growth of *Sargassum* causes significant economic losses in the tourism sector and fishing activities, investing around 210 million dollars to clean the coast (Saldarriaga-Hernandez et al., 2021).

Besides, the massive biomass of *Sargassum* produces severe effects at an ecological level since when *Sargassum* reaches shallow waters, it decomposes, resulting in anoxia and the release of toxic gases, leading to the deaths of marine inhabitants and loss of biodiversity (Saldarriaga-Hernandez et al., 2021). Also, the gases produced during the decomposition of *Sargassum* have a negative impact on the health and well-being of humans, causing neurological, pulmonary, and cardiovascular disorders; and chronic exposure promotes vestibular syndrome and memory loss (Hernández-Bolio et al., 2021).

Thus, to take advantage of the excessive biomass of *Sargassum*, the circular economy has been applied, which implies obtaining high-value products or compounds from waste which can be reused for as long as possible (Sherwood, 2020). For this reason, applying the circular economy approach to formulating *Sargassum*-based aquafeed will mitigate damage to the marine ecosystem and human health. Simultaneously, it will lead to decreased production costs within the aquaculture sector.

Therefore, this work aims to develop the optimal food formulation based on *Sargassum* for shrimp aquaculture. *Sargassum* seaweed collected from the Mexican Caribbean coast and the Gulf of Mexico was the base for performing assays on juvenile shrimps under controlled conditions. After feeding, the final weight (FW), weight gain (WG), specific growth rate (SGR), feed intake (FI), feed conversion rate (FCR), survival, and enzymatic activity were determined and established the potential use of *Sargassum* biomass as an ingredient for shrimp farming.

2. Materials and methods

2.1. Collection of the brown macroalgae samples

Entire individuals of *Sargassum* spp. were collected on February 25th, 2020, in California Beach, La Paz, B.C.S, Mexico, which is situated on the western coast of Cortez Sea (GPS: 24°15′37′N; 110°36′49′W). It was labeled as SG. The sample belonged to the Mexican Caribbean coast, was collected in January 2020 on the east side of Cozumel Island, Punta Sur, Quintana Roo, Mexico (20∘17′ 57.0"N 87∘00′ 22.0"W). The latest sample was labeled as SC. Both samples of the brown macroalgae were immediately rinsed, cleaned off from epiphyte organisms, and stored at −80 °C until further analyses.

Sargassum spp. flours were obtained after a soft oven-dry procedure. Samples were spread in flat aluminum trays and put into a convection oven DKN602C (YAMATO, Santa Clara, CA, USA) at 60 °C for 8 h. The dry samples were pulverized using a conventional Oster brand blender, sieved through an 80-mesh sieve, and used to include in the diets.

2.2. Preparation of crude extracts

Crude extracts of both *Sargassum* flours were obtained through microwave-assisted extraction (MAE) using 1 g of biomass suspended in 50 mL of distilled water. The extraction was performed in triplicates by microwave digestion system MARS 6 (CEM, Charlotte, NC, USA) set at 100 °C for 15 min and stopped in an ice bath. The aqueous extracts were recovered through the Buchner filtration system. Extraction yields are expressed in percentage of dry weight (%DW). The crude extracts were stored at 4 °C until the characterization of protein, carbohydrate, and phenolic contents and the determination of antioxidant properties.

2.3. Chemical characterization and antioxidant profile of Sargassum spp. flour

2.3.1. Ash content

The ash content was determined from the flours by following the laboratory analytical procedure NREL/TP-5100–60956 (Wychen and Laurens, 2013). The results were calculated by weight difference before and after incineration of 3 g of dry biomass for 3 h at 550 °C. Samples were analyzed in triplicate, and the mean values are expressed in % DW.

2.3.2. Total lipid content

The extraction of lipids was carried out by the modified Folch et al. (1957) method as follows. 100 mg of flour was added to 400 μL of water

Table 1
Chemical composition of SG and SC flours.

Chemical content	SG Mean	SC Mean
Ash (% DW)	14.74 ± 1.22*	11.28 ± 0.98
Lipids (mg/g DW)	24.76 ± 1.66	28.16 ± 3.25
Carbohydrates (mg GEQ/g DW)	20.12 ± 4.86	14.10 ± 3.98
Carbohydrates (mg AEQ/g DW)	84.70 ± 20.43	59.39 ± 16.78
Proteins by Bradford (mg/g DW)	7.12 ± 2.13	8.77 ± 2.75*
Proteins by Lowry (mg/g DW)	65.14 ± 13.10	135.53 ± 38.50
Phenolics (mg PEQ/g DW)	4.83 ± 1.15	4.07 ± 1.24
Antioxidant by FRAP (μmol TE/ g DW)	76.08 ± 51.64	95.1 ± 23.11*
Antioxidant by DPPH (μmol TE/ g DW)	5.77 ± 1.99	13.98 ± 2.42*

Sargassum sp. from Gulf of California (SG), *Sargassum* spp. from Mexican Caribbean coast (SC), dried weight (DW), glucose equivalent (GEQ), alginate equivalent (AEQ), phloroglucinol equivalent (PEQ), Trolox equivalent (TE), ferric reducing ability of plasma (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), * statistical significance (t-test, $p \leq 0.05$).

Table 2
Ingredient composition and proximal analysis of experimental diets.

Ingredients (g/kg diet)	Experimental diets				
	Control	SC2.5	SC5	SG2.5	SG5
Fish meal[a]	228	228	228	228	228
Soybean meal[b]	330	330	330	330	330
Wheat meal[c]	355	330	305	330	305
Fish oil[a]	30	30	30	30	30
Soy lecithin[d]	40	40	40	40	40
Alginic acid[e]	10	10	10	10	10
Vitamin-mineral premix[f]	5	5	5	5	5
Vitamin C[g]	1	1	1	1	1
Choline Chloride[e]	1	1	1	1	1
Sargassum spp. *(Caribbean)*	0	25	50	0	0
Sargassum sp. (Gulf of California)	0	0	0	25	50
Proximal composition (% on dry base)					
Protein	37.1 ± 0.04	37.1 ± 0.09	37.1 ± 0.06	36.9 ± 0.04	37.0 ± 0.03
Lipids	8.61 ± 0.07	9.39 ± 0.03	9.45 ± 0.06	9.36 ± 0.03	9.45 ± 0.03
Crude fiber	0.93 ± 0.06	0.96 ± 0.06	1.13 ± 0.06	0.93 ± 0.06	1.40 ± 0.10
Ash	7.89 ± 0.02	8.47 ± 0.03	8.83 ± 0.02	8.70 ± 0.01	9.43 ± 0.01
NFE[h]	45.52	44.21	43.48	44.12	42.76

Proximal composition values are given as mean ± SD of triplicate determinations.
[a] PMA S.A. de C.V., Jalisco, MX.
[b] PIASA S.A. de C.V., Baja California Sur, MX.
[c] Molino San Cristobal, Sonora, MX.
[d] Suministros AZ, Baja California Sur, MX.
[e] Sigma Aldrich, St. Louis, Missouri, US.
[f] Vitamin-mineral premix: detailed content in Peña-Rodríguez et al. (2020)
[g] Prilabsa, Sinaloa, MX
[h] Nitrogen-free extract, estimated by difference.

and 6 mL of a mixture of chloroform from J.T. Baker (Phillipsburg, NJ, USA) and methanol from Sigma–Aldrich® (St. Louis, MO, USA) (2:1 *v/v*) then the mixture was vortexed. The samples were sonicated for 10 min and mixed for 30 min at 150 rpm. The extract was centrifuged at 2000 rpm for 10 min, and the supernatant was collected for further analysis.

The procedure to determine the total lipid content was carried out with 100 μL of the treated sample mixed with 2 mL of sulfuric acid from J.T. Baker (Phillipsburg, NJ, USA) and placed into a digester (HACH, Dusseldorf, Germany) for 10 min at 100 °C. After that, the tubes were cooled on an ice bath for 5 min, and 5 mL of phospho-vanillin reagent from Sigma–Aldrich® (St. Louis, MO, USA) was added, after vortexed the samples were incubated at 37 °C for 15 min, with an agitation rate of 200 rpm. The absorbance was measured at 530 nm in a DR 5000™ UV-Vis Spectrophotometer (HACH, Düsseldorf, Germany). The lipid content was determined with a standard curve of a commercial extra virgin olive oil (Nutrioli®, Monterrey, NL, Mexico) ($y = 0.0078x-0.012$, $R^2 = 0.977$). Samples were measured by triplicate; the results and corresponding mean values are expressed in the weight of lipids per gram of flour (mg/g DW).

Table 3
Weight gain (WG) specific growth rate (SGR), feed intake (FI), feed conversion rate (FCR), and Survival of *L. vannamei* after 35 days of feeding with diets containing *Sargassum* spp.

Treatments	WG (g)	SGR (%/day)	FI (g)	FCR	Survival (%)
Control	2.64 ± 0.04[a]	4.43 ± 0.04[a]	4.37 ± 0.20[a]	1.65 ± 0.05	97 ± 6
SC2.5	2.91 ± 0.08[b]	4.65 ± 0.07[b]	4.68 ± 0.04[b]	1.61 ± 0.05	97 ± 6
SC5	2.83 ± 0.08[b]	4.59 ± 0.07[b]	4.69 ± 0.15[b]	1.66 ± 0.03	97 ± 6
SG2.5	2.93 ± 0.05[b]	4.67 ± 0.04[b]	4.82 ± 0.07[b]	1.64 ± 0.05	90 ± 10
SG5	2.87 ± 0.03[b]	4.62 ± 0.02[b]	4.93 ± 0.02[b]	1.72 ± 0.01	100

Average initial weight 0.71 ± 0.03 g
Values are given as mean ± SD of triplicate determinations. Superscripts in columns indicate homogeneous subsets determined by Tukey's test.
Survival (%) = Final number of shrimp/initial number shrimp x100.
WG (g) = Final weight - Initial weight.
SGR (%/day) = 100 x (ln FW−ln Initial weight)/number of days.
FI (g) = Total of feed provided per shrimp during feeding trial
FCR = FI/(FW- Initial weight).

2.3.3. Total protein content

Soluble proteins were quantified in the crude extracts by Bradford (1976) assay and with a modified Lowry protein assay that follows the Lowry et al. (1951) principle. The microplate procedure for the Bradford assay was performed by pipetting 10 μL of appropriate standards Bovine Serum Albumin (BSA) (PROMEGA, Madison, WI, USA) and samples into a 96-well microplate then mixed with 200 μL of Bradford reagent from Sigma–Aldrich® (St. Louis, MO, USA). The microplate was incubated at room temperature (RT) for 5 min, and the measurements were made on a BioTek Synergy HTX microplate spectrophotometer (Agilent, Santa Clara, CA, USA) at a wavelength of 595 nm.

For the modified Lowry protein assay, the procedure used by Saldarriaga et al. (2020) was followed, using 40 μL of standards, and samples were placed into a 96-well microplate and mixed with 200 μL of the modified Lowry reagent from Thermo Scientific (N.L., Mexico). The microplate was incubated for 10 min at RT, adding 20 μL of the 1X Folin-Ciocalteu reagent from Sigma–Aldrich® (St. Louis, MO, USA) into each well. After mixing, the plate was incubated at RT for 30 min, and the absorbance was measured at 750 nm on a BioTek Synergy HTX microplate spectrophotometer (Agilent, Santa Clara, CA, USA).

The soluble protein concentration for each procedure was determined by a standard curve of BSA (PROMEGA, Madison, WI, USA) for Bradford ($y = 0.98x+0.0308$, $R^2 = 0.962$) and Lowry ($y = 0.0007x+0.0592$, $R^2 = 0.966$) assays. All samples were analyzed in triplicate, and the results are expressed as protein weight per gram of flour (mg/g DW).

2.3.4. Total carbohydrate content

The carbohydrate content of the *Sargassum* spp. flours were determined by the sulfuric acid method proposed by López-Legarda et al. (2017). In brief, a 0.3 mL sample was mixed with 1 mL of sulfuric acid from J.T. Baker (Phillipsburg, NJ, USA). The reaction was stopped with ice for 2 min, and the absorbance was measured on a microplate with 200 μL of the treated sample at 315 nm on a BioTek Synergy HTX

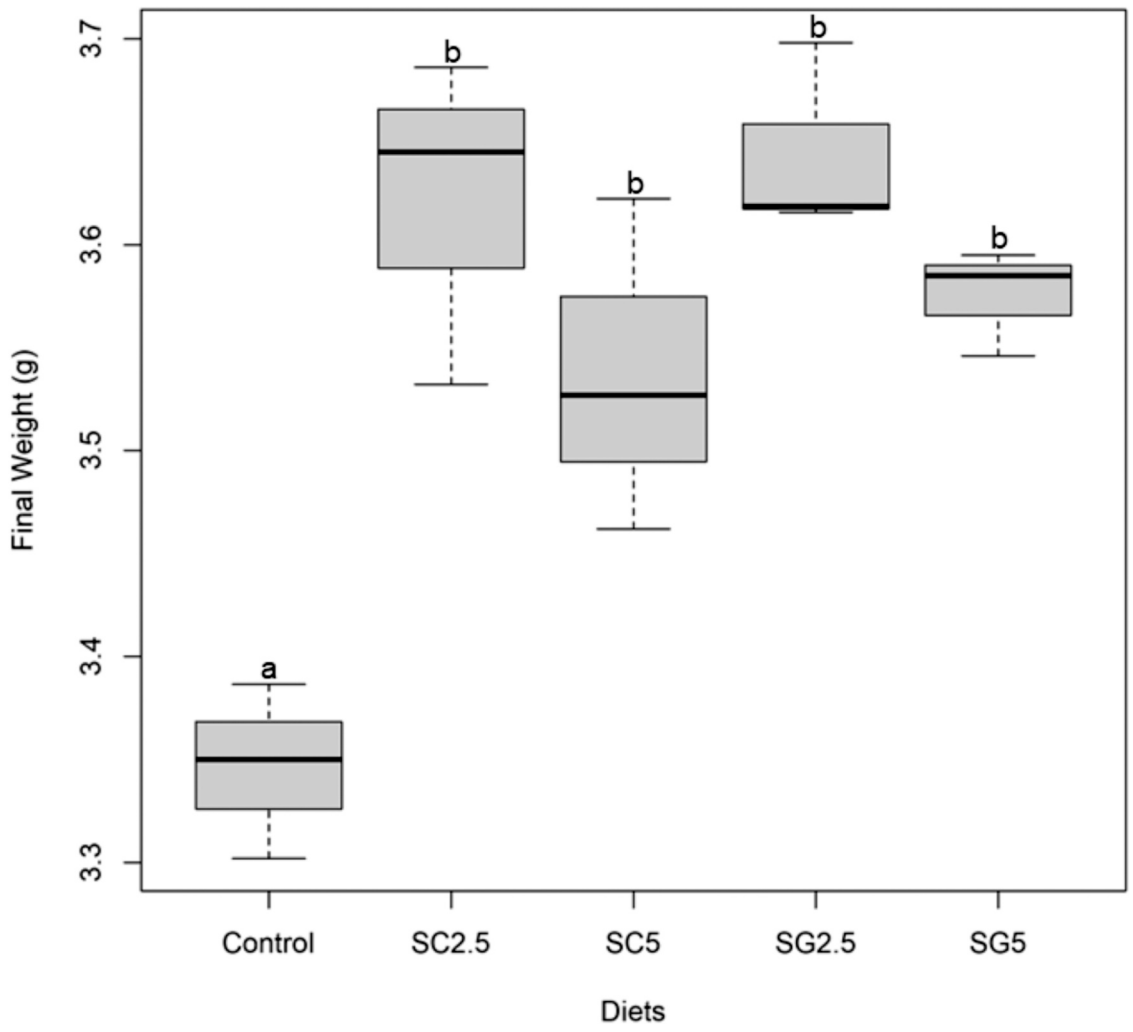

Fig. 1. Final weight (g) of shrimp (*Litopenaeus vannamei*) fed for 35 days with the reference diet formulation (Control) and the diets formulated with 2.5 % and 5 % of *Sargassum* spp. from the Caribbean (SC2.5 and SC5) and *Sargassum sp.* from Gulf of California (SG2.5 and SG5).

microplate spectrophotometer (Agilent, Santa Clara, CA, USA). The determination of polysaccharide content in samples was calculated with a standard curve of glucose ($y = 5.246x - 0.0011$, $R^2 = 0.993$), using a concentration range from 0 to 200 mg/mL and with a standard curve of alginate ($y = 1.2472x - 0.0025$, $R^2 = 0.929$) with a concentration range from 0 to 100 mg/mL, both from Sigma–Aldrich® (St. Louis, MO, USA). The samples were analyzed in triplicate, and the results are reported as the weight of the equivalent (glucose or alginate) per gram of flour (mg G/A EQ/g DW).

2.3.5. Total phenolic content

The Folin-Ciocalteu method to determine the total phenolic content (TPC) based on the Singleton et al. (1965) procedure was adapted to the microplate. The assay was started with 20 μL of extracts and a corresponding standard phloroglucinol from Sigma–Aldrich® (St. Louis, MO, USA), then mixed with 10 μL of Folin-Ciocalteu reagent (Sigma–Aldrich®, St. Louis, MO, USA), 60 μL MiliQ Water, and 110 μL of sodium carbonate (7.5 %). The microplate was agitated and incubated at RT in the dark for one hour. The absorbance was measured at 730 nm on a BioTek Synergy HTX microplate spectrophotometer (Agilent, Santa Clara, CA, USA). The TPC content was calculated with a standard curve of phloroglucinol Sigma–Aldrich®, St. Louis, MO, USA) ($y = 6.756x + 0.0172$, $R^2 = 0.998$) using a concentration range from 0.025 to 0.100 mg/mL. All samples were analyzed in triplicate, and the results are expressed as the weight of phloroglucinol equivalent per gram of flour (mg PEQ/g DW).

2.3.6. Antioxidant properties

Free radical scavenging capacity was evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay according to Brand-Williams et al., 1995 and by the ferric reducing ability of plasma (FRAP) assay taken from Benzie and Strain, 1996.

2.3.6.1. DPPH. For the assay of the DPPH, the protocol proposed by Brand-Williams et al. (1995) was followed. The DPPH solution was prepared using 5 mg of DPPH Sigma–Aldrich® (St. Louis, MO, USA) and 200 mL of absolute ethanol (CTR Scientific, N.L, Mexico), stirred, and incubated for 3 h in the dark. The standard curve was prepared from Trolox, Sigma–Aldrich® (St. Louis, MO, USA), using a concentration range of 0.5–100 ppm. The readings were taken in a BioTek Synergy HTX microplate spectrophotometer reader (Agilent, Santa Clara, CA, USA) at a wavelength of 540 nm, using 20 μL of the *Sargassum* samples or positive controls, and 180 μL of the DPPH solution in microplates of 96 wells; prior to taking the readings, the microplates were incubated for one hour in dark conditions. Antioxidant capacity was reported in Trolox equivalent according to the standard curve ($y = 0.0032x + 0.0018$, $R^2 = 0.9716$).

2.3.6.2. FRAP. For the FRAP assay, the methodology reported by Benzie and Strain, 1996 was followed. FRAP reagent was prepared from

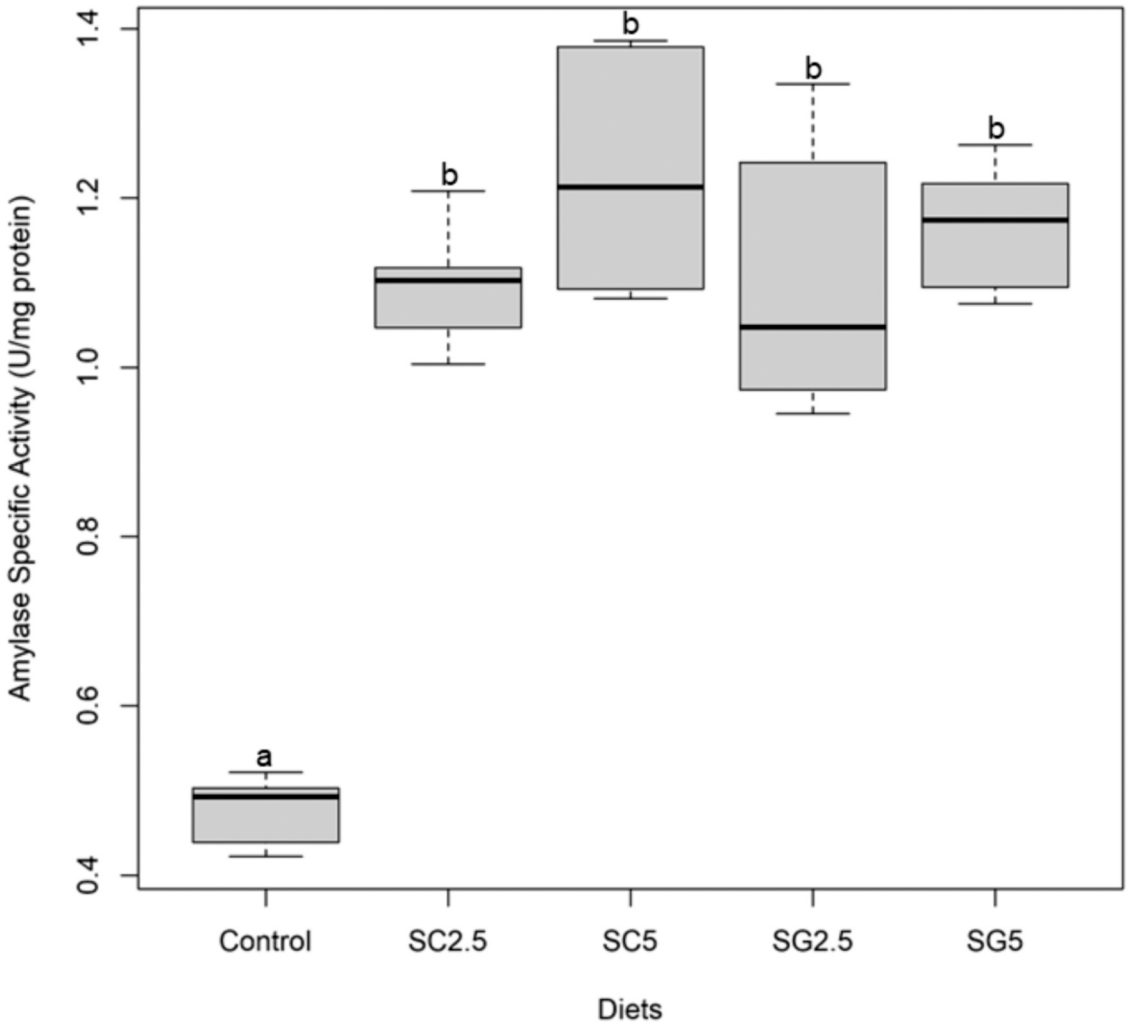

Fig. 2. Amylase specific activity (U/mg protein) of hepatopancreas extracts from shrimp (*Litopenaeus vannamei*) fed for 35 days with the reference diet formulation (Control) and the diets formulated with 2.5 % and 5 % of *Sargassum* spp. from the Caribbean (SC2.5 and SC5) and *Sargassum sp.* from Gulf of California (SG2.5 and SG5).

a 300 mM acetate buffer at pH 3.6, which contains sodium acetate Sigma–Aldrich® (St. Louis, MO, USA), acetic acid, a solution of tris (2-pyridyl)-s-triazine (TPTZ) Sigma–Aldrich®, (St. Louis, MO, USA), and a solution of iron chloride (Fermont, NL, Mexico). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), Sigma–Aldrich®, (St. Louis, MO, USA) a concentration range from 0.025 to 1 mg/mL were used to develop the standard curve. The antioxidant capacity of the *Sargassum* samples and the standard curve was measured in a BioTek Synergy HTX microplate spectrophotometer reader (Agilent, Santa Clara, CA, USA) at a wavelength of 593 nm after incubating the 96-well microplate for 4 min, using 5 μL of the *Sargassum* extract or positive controls with Trolox Sigma–Aldrich®, (St. Louis, MO, USA) and 150 μL of the FRAP reagent. The antioxidant activity in the samples was calculated from the standard curve of Trolox ($y = 1.7535x + 0.077$, $R^2 = 0.9872$). All samples were performed in triplicate.

2.4. Feeding bioassay

2.4.1. Feed formulation

Five experimental diets were formulated; two diets with 2.5 % and 5 % inclusion of *Sargassum* spp. from the Mexican Caribbean coast (SC2.5 and SC5), *Sargassum sp.* from Gulf of California (SG2.5 and SG5), and one reference diet as control without seaweed inclusion (Table 1). In the case of seaweed diets, the inclusion level was in replacement of wheat meal. The diets were manufactured by mixing all dry ingredients in a 1.5-L Kitchen-Aid™ mixer (Benton Harbor, MI, USA) to obtain a homogeneous mixture, then the oil-based ingredients and water (~30 % of the mix weight) was added and mix to obtain a consistent paste. The mixture was passed through a 2 mm die in a ½ hp Torrey® meat grinder. The pellets were dried in a forced-air oven at 45 °C for 12 h and stored at 4 °C until further use.

The proximal composition of the whole meal and pre-digested macroalgae formulations were analyzed according to reported methods to analyze crude protein (Dumas combustion method, Ebeling, 1968), lipids (Method 2003.05; AOAC, 2005), ash (Method 942.05; AOAC, 2005) and crude fiber (Method 978.10; AOAC, 2005). Nitrogen-free extract (NFE) was estimated on a dry weight basis by subtracting the percentages of crude proteins, lipids, ash, and crude fiber from 100 %.

2.4.2. Feeding trial

A 35-day feeding trial was conducted using juvenile *Litopenaeus vannamei* to evaluate the experimental diets per triplicate as described by (Omont et al., 2019) with some modifications. Each replicate consisted of a 60 L fiberglass tank, with 10 shrimps randomly distributed (initial average weight of 0.71 ± 0.03 g), provided with filtered marine water (through 1-μm mesh and sterilized with UV light) at 38.1 ± 0.1 and pH 8.0 ± 0.13, constant aeration (dissolved oxygen (DO) > 5 mg/L), and controlled photoperiod (12 h of light after 12 h of dark) and

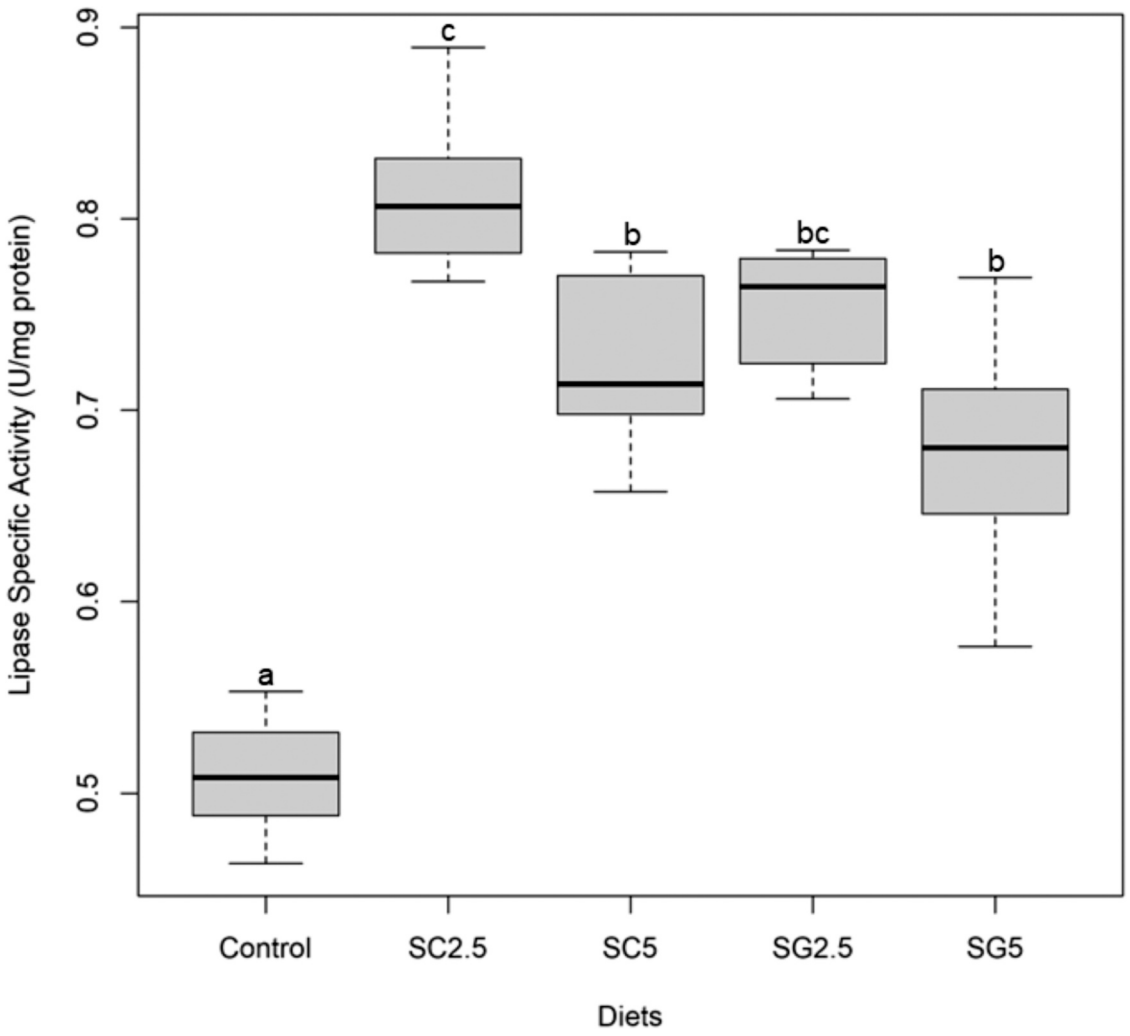

Fig. 3. Lipase specific activity (U/mg protein) of hepatopancreas extracts from shrimp (*Litopenaeus vannamei*) fed for 35 days with the reference diet formulation (Control) and the diets formulated with 2.5 % and 5 % of *Sargassum* spp. from the Caribbean (SC2.5 and SC5) and *Sargassum sp.* from Gulf of California (SG2.5 and SG5).

temperature (28.3 ± 0.3 °C). Shrimp were fed *ad libitum* with an initial rate of 8 % shrimp biomass divided into two rations (at 09:00 and 14:00 h). After day 2, feeding rates were adjusted according to consumption. Every morning (08:00 h), each experimental tank was siphoned to remove unconsumed feed, molts, and feces, and 60 % of water exchange was made.

At the end of the feeding trial period, shrimp performance was estimated in terms of final weight (FW), weight gain (WG), specific growth rate (SGR), feed intake (FI), feed conversion rate (FCR) and survival (see details in Table 3).

2.5. Enzymatic activity

For this experiment, nine individuals from each treatment described above (three per experimental tank) were sampled, and the hepatopancreas was removed and stored at −80 °C until their use to determine the activity of digestive enzymes.

Soluble proteins from hepatopancreas were extracted according to the methodology proposed by Omont et al. (2019). The hepatopancreas was homogenized with a buffer at pH 7.5 prepared with Tris HCl (50 mM) Sigma–Aldrich®, (St. Louis, MO, USA) and $CaCl_2$ (20 mM) (1:5; w: v) Sigma–Aldrich®, (St. Louis, MO, USA), using FastPrep-24™ MP Biomedicals, (Fort Lauderdale, FL, USA). Homogenates were centrifuged for 3 min at 3000 rpm and 4 °C to recover supernatants and prepare dilutions for enzymatic activity evaluations.

The hydrolytic activity of amylase was evaluated on soluble starch (Vega-Villasante et al., 1993). Lipase activity was assayed using *β*-naphthyl caprylate (BNC), Sigma–Aldrich® (St. Louis, MO, USA) as described by Nolasco-Soria et al. (2018), trypsin activity was determined on Na-benzoyl-DL-arginine 4-nitroanilide hydrochloride, (BAPNA) Sigma–Aldrich® (St. Louis, MO, USA) substrate (Erlanger et al., 1961), and chymotrypsin activity was measured using *N*-succinyl-Ala-Ala-Pro-Phe p-nitroanilide (SAAPNA), Sigma–Aldrich® (St. Louis, MO, USA) according to Del Mar et al. (1979). The total protein content of the extracts, determined according to Bradford (1976), was used to normalize the specific enzyme activity which is expressed in U/mg protein. The unit (U) is the enzyme amount required to increase absorbance by 0.01 per minute. All methods were performed at the microscale using microplate spectrophotometer Multiskan FC (Thermo Scientific).

2.6. Data analysis

Statistical analyses were carried out using R programming language (R Core Team, 2022) version 4.1.1. All data were checked for normality and homoscedasticity with the Shapiro-Wilk and Bartlett tests, respectively. Accordingly, parametric or non-parametric analyses of variance followed by appropriate post-hoc tests were run to compare the

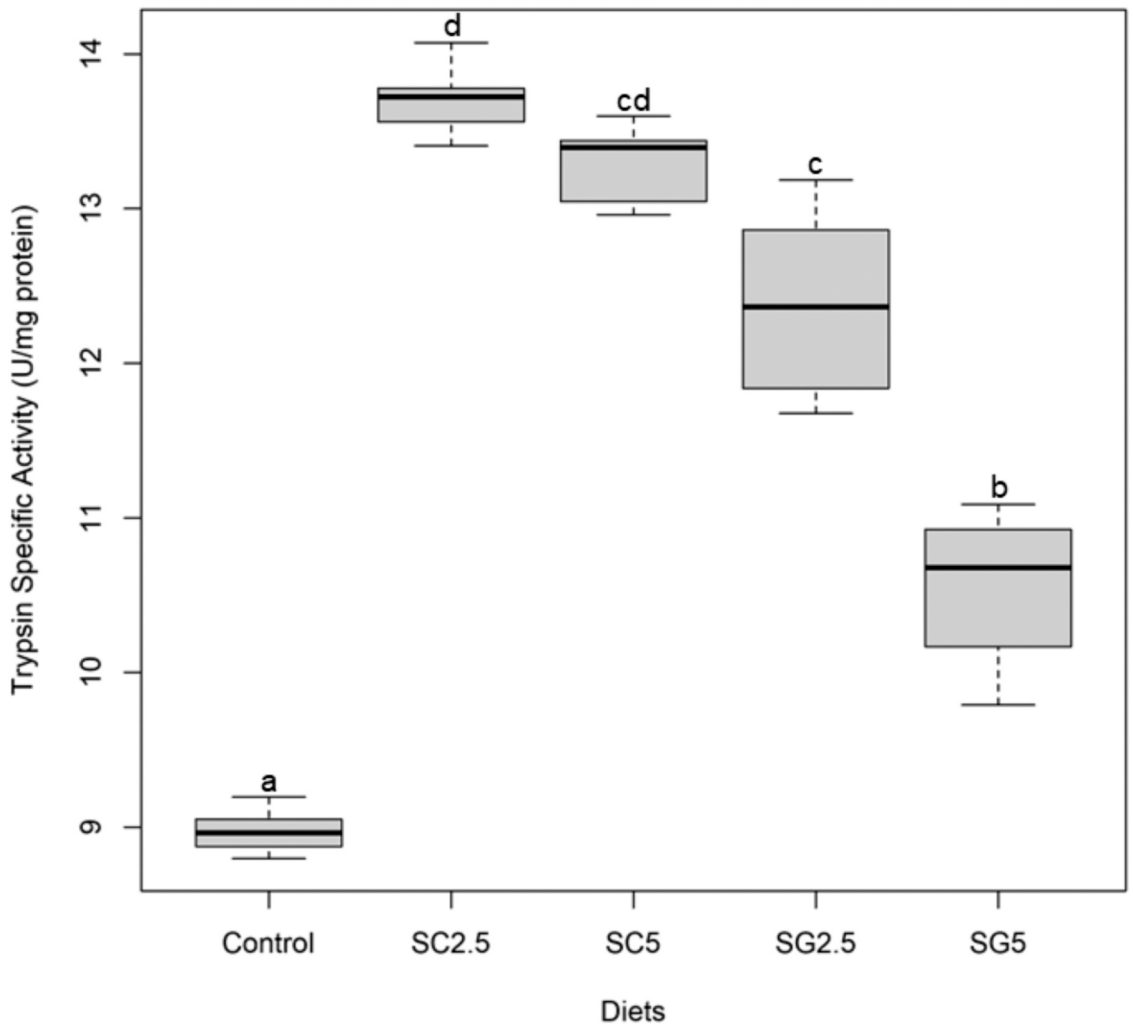

Fig. 4. Trypsin specific activity (U/mg protein) of hepatopancreas extracts from shrimp (*Litopenaeus vannamei*) fed for 35 days with the reference diet formulation (Control) and the diets formulated with 2.5 % and 5 % of *Sargassum* spp. from the Caribbean (SC2.5 and SC5) and *Sargassum sp.* from Gulf of California (SG2.5 and SG5).

chemical contents of the *Sargassum* flours and to evaluate the effect of dietary treatments on enzyme activities. The statistical tests were performed with at least $\alpha = 0.05$.

3. Results and discussion

3.1. Chemical characterization and antioxidant profile of Sargassum Flours

Sargassum has presented great interest due to its high nutritional content for its application in aquaculture. Therefore, it is essential to identify the chemical composition and value components since these vary depending on the geographical region and season of the year (Amador-Castro et al., 2021; Saldarriaga-Hernandez et al., 2020; Silva et al., 2021).

In that sense, the chemical composition (content of ashes, lipids, carbohydrates, proteins, phenols, and antioxidants) of *Sargassum* flour from the Gulf of California and the Caribbean of Mexico is shown in Table 1. Observed results indicated that the SG presents a higher percentage of ash of 14.74 % DW with respect to the SC of 11.28 % DW. This is consistent with the 9.5 % DW of ash previously reported by Oyesiku and Egunyomi (2015) for the species *Sargassum natans* and *Sargassum fluitans* collected in Nigeria. However, these authors differ from Saldarriaga-Hernandez et al. (2021) (24–30 % DW) for *Sargassum* spp. from the Mexican Caribbean coast in the months of May and September. Ashes are the least desired components in *Sargassum* since they are made up of inorganic matter, affecting the chemical composition of *Sargassum* and the effectiveness of pre-treatments to obtain carbohydrates or other compounds of value (Kenney et al., 2014).

While in the analysis of total lipids, averages of 24.76 mg/g of DW (2.476 % DW) for SG and 28.16 mg/g of DW (2.816 % DW) for SC, and no significant difference (NS, $p > 0.05$) was observed between the samples (Table 1). These results are consistent with the range of 0.01–13.7 % DW of lipids present in *Sargassum*. The lipids are high-value compounds in *Sargassum* since they have unsaturated fatty acids, and the variations in percentages of lipids may be due to seasons of the year since winter correlates with an increase in the lipid production (Saldarriaga-Hernandez et al., 2020).

Carbohydrates are a source of energy and play important roles as immune system stimulants, anticoagulants, anti-inflammatories, and antivirals (Rioux and Turgeon, 2015). Two comparisons were made, the glucose and alginate equivalent weight samples, presenting the mean glucose weight equivalent samples of 20.12 mg GEQ/g DW for SG and 14.10 mg GEQ/g DW for SC, with a $p > 0.05$ (Table 1). The alginate equivalent weight samples showed means of 84.70 mg AEQ/g DW and 59.39 mg AEQ/g DW for SG and SC, respectively, and NS ($p > 0.05$) was observed between the samples (Table 1). The results agreed with the percentage of carbohydrates from 1.80 % to 7.18 % DW of *Sargassum*

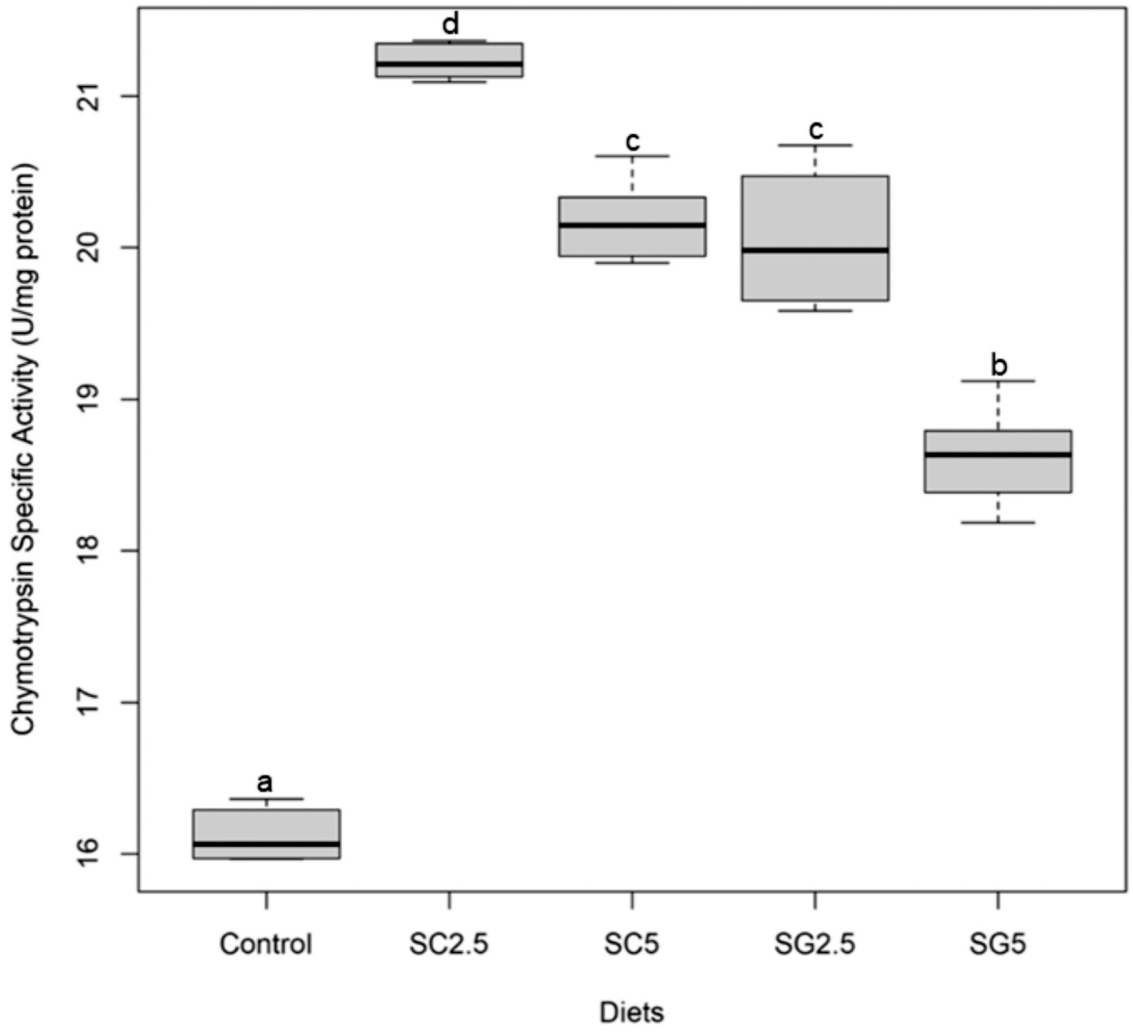

Fig. 5. Chymotrypsin specific activity (U/mg protein) of hepatopancreas extracts from shrimp (*Litopenaeus vannamei*) fed for 35 days with the reference diet formulation (Control) and the diets formulated with 2.5 % and 5 % of *Sargassum* spp. from the Caribbean (SC2.5 and SC5) and *Sargassum sp.* from Gulf of California (SG2.5 and SG5).

spp. from the Mexican coast (Saldarriaga-Hernandez et al., 2021), but these contrast with Oyesiku et al. (2015) since they indicate that *Sargassum* spp. collected in Nigeria has a concentration of 5.73 mg/g DW. These variations in the concentration of carbohydrates may be due to the extraction method of *Sargassum*, temporality, and geography of sample collection (Saldarriaga-Hernandez et al., 2021).

As for proteins that provide amino acids necessary for the construction of the body of any species (Oyesiku and Egunyomi, 2015), they represent from 3 % to 17 % DW of pelagic *Sargassum* (Amador-Castro et al., 2021a). Which agrees with the protein concentration using the Lowry and Bradford methods. Lowry's assay indicated a protein concentration of 65.14 mg/g DW (6.14 % DW) for SG and 38.50 mg/g DW (3.85 % DW) for SC, with a $p > 0.05$ (Table 1). However, it differs from the protein content using the Bradford method with 7.12 mg/g DW (0.712 % DW) for SG and 2.75 mg/g DW (0.275 % DW) for SC, presenting significant differences with a $p \leq 0.05$ (Table 1). These variations are due to the basis of the technique to identify proteins. In the first case, Lowry's method depends on the presence of tryptophan and tyrosine residues to form complexes with copper, which are the main substrates of chymotrypsin, being catalyzed by cutting the peptide bonds of the adjacent carboxyl groups of aromatic amino acids, which plays an important role in the digestive system and the immune response (Castellanos-Ochoa et al., 2022; Gong et al., 2014). While Bradford's method measures the binding of Coomassie Brilliant Blue G-250 with proteins, allowing to know the total protein content in *Sargassum* sample (Edelson and Robert, 1981).

Another interesting component is phenols, which have antioxidant, antimicrobial, anti-inflammatory, and anticancer activities and significantly impact the daily diet (Zhong et al., 2020). The total phenolic content in this study for SG and SC showed similar concentrations of 4.83 mgPEQ/g DW (0.483 % DW) and 4.07 mgPEQ/g DW (0.407 % DW), respectively, with a $p > 0.05$, which agrees with what was reported by Praiboon et al. (2018), since they obtained concentrations of 1.55–4.97 mg/g DW of total phenol content in samples of *Sargassum oligocystum*, but differs with the percentage of the total phenolic content of *Sargassum vulgare* (0.56–3.61 %) and the concentration of *Sargassum* obtained from the Mexican Caribbean coast (1.5–2.31 mg GA/g DW) (Plouguerné et al., 2012; Saldarriaga-Hernandez et al., 2021). These differences in the concentration of total phenolic content can be explained by exposure to UV radiation, the salinity of the water, and the type of extraction to obtain phenols (Saldarriaga-Hernandez et al., 2021).

In terms of antioxidant properties, the results presented a significant difference ($p \leq 0.05$) between the SG and SC samples (using the FRAP and DPPH methods). The FRAP method indicated that the SC presented a higher concentration of antioxidants (95.1 μmol TE/g or 23.80 mg TE/g DW) with respect to the SG (76.08 μmol TE/ g DW or 19.02 mg TE/g DW). These results are consistent with the ranges of antioxidant

concentration for *Sargassum muticum* (4.18–18.33 mg AAE/g DE) (Silva et al., 2021) but contrast with the antioxidant concentration from the lipid-soluble fraction of *Sargassum cristaefolium* (132.06 μmol FE/ g DW - 688.09 μmol FE/g dry extract) (Saraswati et al., 2020). According to the DPPH method, the antioxidant concentration was lower than the FRAP method. SC sample showed a higher concentration of antioxidants (13.98 μmol TE/ g DW), which agrees with Saraswati et al. (2020) for samples *of Sargassum cristaefolium* (11.94–67.15 mg TE/g dry extract), differing from our SG samples (5.77 μmol TE/ g DW). The variations observed are probably caused by the solvent used for the extraction of *Sargassum*, as well as the mechanism as the antioxidants act in the assay since the FRAP assay measures the reduction potential under acidic conditions by electro-donation and reducing the yellow ferric-TPTZ complex to the blue Ferrous - TPTZ complex. While the DPPH test consists of the reduction of the DPPH radical in the presence of an antioxidant compound that donates hydrogen electrons (Silva et al., 2021).

In summary, the SG showed a high percentage of ash. In comparison, the SC presented a higher content of proteins in the Bradford assay and higher antioxidants content by FRAP and DPPH assays, with a $p \leq 0.05$. No significant differences were observed between the SG and SC regarding the content of carbohydrates, phenols, and lipids.

3.2. Experimental on shrimps

3.2.1. Feed formulation

The composition of seaweed had an evident effect on the experimental diets, with an increase in total lipids, crude fiber, and ash (Table 2). On the other hand, protein content remained similar among all diets. In the present study, seaweed meal was included in the replacement of wheat meal, which provides a lower amount of lipids, fiber, and minerals to the diet than seaweed (Amoriello et al., 2021). The study considered two inclusion levels, 2.5 %, and 5 %, for each sample of *Sargassum*.

3.2.2. Growth performance

After 35 days of bioassay, all treatments showed a shrimp survival rate ≥90 % ($p > 0.05$). (Table 3). Seaweed-based diets resulted in a significant increase in shrimp growth in terms of final weight (Fig. 1), weight gain (WG), and specific growth rate (SGR) compared to the control treatment ($p < 0.05$). Furthermore, no significant differences in growth among the *Sargassum* species or inclusion levels in the diets were shown.

The seaweed content in the pelleted feed varied among studies; nevertheless, when the whole meal is used, inclusion levels higher than 10 % usually affects shrimp performance (Rodríguez-González et al., 2014; Serrano, Santizo, and Tumbokon, 2015) suggesting not to exceed 5 % to obtain beneficial properties of seaweed for shrimp health and growth (Cruz-SuÁrez et al., 2009; Elizondo-González et al., 2018; Yu et al., 2016). Low inclusion levels could promote a better growth rate, as in *L. vannamei* fed with *Sargassum polycystum* included at 500 mg/kg of feed, showing higher dietary protein utilization (Abdel-Rahim et al., 2021a). In the same way, the 0.5 % inclusion level of brown seaweeds (*Sargassum filipendula* or *Undaria pinnatifida*) increased the gut epithelial surface in *L. vannamei*, associated with higher nutrient absorption (Schleder et al., 2018). According to Gamboa-Delgado et al. (2011), carbon and nitrogen from seaweed (*Ulva clathrata*) can be efficiently incorporated into the shrimp muscle at higher rates than commercial balanced feed. Seaweed inclusion in the feed may also promote better health or immune response in shrimp (Gamboa-Delgado et al., 2011). The inclusion of *Sargassum cristaefolium* meal, between 0.5 % and 4 % in feed, showed improved hematological parameters and reduced *Vibrio* counts in the intestine in *L. vannamei* (Jahromi et al., 2021). In the case of hot water extracts of *Sargassum* species, an improvement of growth and immune protection when included at 0.5 % in the *L. vannamei* diet (Lee et al., 2020) or when used to enrich artemia to feed *Penaeus monodon* post-larvae (Immanuel et al., 2010).

Regarding feed intake, the *Sargassum* diets promoted it with respect to the control diet without a negative effect on feed conversion rate (FCR). Dietary inclusion in a range of 1–8 % of *Sargassum horneri* significantly increased feed palatability by reducing the relative time of consumption (Gunho et al., 2020). In the fish *Paralichthys olivaceus*, the use of *Sargassum fulvellum* induced higher feed intake, attributed in part to the presence of compounds with attractant properties such as sulphated polysaccharides and betaine (Ragaza et al., 2021). Moreover, crude extracts from *Eisenia sp*, *Ulva sp.,* and *Porphyra sp.* included in the feed have promoted higher feed intake in *L. vannamei* shrimp (Omont et al., 2019).

On the other hand, some studies point out excessive dietary supplementation of seaweed in feed can also increase the viscosity of intestinal contents due to the soluble non-starch polysaccharide content. This increased viscosity can lead to reduced nutrient digestibility and decreased protein and lipid digestibility, resulting in reduced growth performance (Niu et al., 2015, 2018).

3.2.3. Digestive performance

The digestive performance of shrimp fed during 35 days with formulated diets with *Sargassum* flours was evaluated through the specific activity of amylase, lipase, trypsin, and chymotrypsin. For all digestive enzymes studied, their specific activities were significantly increased by the inclusion of *Sargassum* in diets compared to the reference diet (Kruskall-Wallis test, $p < 0.05$).

For amylase activity, the pairwise analysis revealed no significance between the treatments with a specific activity ranging from 1.097 ± 0.07 (SC2.5) to 1.227 ± 0.13 U/mg protein (SC5) with the highest activity registered for the diet with 5 % of *Sargassum* spp. from the Caribbean (Fig. 2).

The shrimp's digestive enzymatic performance is related to the digestibility of the available nutrients. It has been shown that the addition of some brown seaweeds like *Sargassum filipendula* and *Undaria pinnatifida* in shrimp feed affected the amylase activity but had no effect on the growth of shrimp performance (Schleder et al., 2018). In contrast, in the present work, it was found that the addition of all inclusion levels of *Sargassum sp.* from the Gulf of California (SG 2.5 %, SG 5 %) made more relevant effects on growth shrimp than *Sargassum sp.* (Cruz-SuÁrez et al., 2009; Omont et al., 2021). Related to the chemical composition, brown seaweeds are known for their highly digestible carbohydrate content (Olsson et al., 2020). For this reason, the inclusion of the *Sargassum* flours into the diets (SG 2.5 %, SG 5 %, SC 2.5 %, SG 5 %) can contribute to the increasing activity of amylase and a better absorption of the feeds.

The highest lipase specific activity was observed for the treatment with 2.5 % of *Sargassum* spp. from the Caribbean (SC2.5: 0.814 ± 0.04 U/mg protein), although it was not significant compared to SG2.5 (0.754 ± 0.03 U/mg protein), it was significantly higher than the two treatments with 5 % of *Sargassum* spp.; SC5 (0.723 ± 0.05 U/mg protein) and SG5 (0.677 ± 0.06 U/mg protein) (Dunn test, $p < 0.05$) (Fig. 3).

On the other hand, regarding lipase activity, results show that the inclusion of 2.5 % of *Sargassum* spp. from the Caribbean (SC2.5) or *Sargassum sp.* from the Gulf of California (SG2.5) contributes to a better intake of the lipids included in the diet, that can be noticed with the final weight performance (Fig. 1), compared with the other treatments. On the contrary, Omont et al. (2019) found that the addition of predigested *Eisenia sp.,* another brown seaweed, did not show an increase of lipase activity when included in 5 %, 10 %, and 15 %. Based on this, our finding demonstrated that the addition of a lower amount of *Sargassum sp.* contributes to better feed digestion.

The specific activities of trypsin (Fig. 4) and chymotrypsin (Fig. 5) showed similar trends. The trypsin activity was significantly enhanced by the SC incorporation into the diet compared to SG. No significance was found between the two inclusion levels of *Sargassum* from the Caribbean, with 13.711 ± 0.23 and 13.306 ± 0.25 U/mg protein for

SC2.5 and SC5, respectively. In contrast, the lowest inclusion level of *Sargassum sp.* from the Gulf of California rather increased trypsin activity (SG2.5, 12.383 ± 0.61 and SG, 10.554 ± 0.49 U/mg protein).

The chymotrypsin specific activity was significantly higher (21.226 ± 0.11 U/mg protein) with the lowest inclusion level of *Sargassum* spp. (SC2.5) compared to the treatments SC5 (20.181 ± 0.26 U/mg protein) and SG2.5 (20.058 ± 0.05 U/mg protein) and SG5 (18.626 ± 0.33 U/mg protein) (Fig. 5).

Trypsin and chymotrypsin in shrimp are responsible for the digestion of total proteins (Galgani, Benyamin, and Ceccaldi, 1984). Both proteases have similar trends in this work, indicating that both *Sargassum* species share quality and quantity protein content (Table 1). Nonetheless, a lower inclusion level of *Sargassum* spp. from the Caribbean is a better treatment.

As other authors have reported, the hydrolytic digestive enzyme activity could provide evidence of the beneficial prebiotic effect of *Sargassum*-based diets on shrimp growth. The prebiotic properties of the seaweed likely stimulate the growth of endogenous probiotic bacteria in the shrimp gut, which in turn leads to increased secretion of exogenous hydrolytic enzymes. This heightened enzyme activity results in more efficient digestion of ingested foods, improving growth performance (Muttharasi et al., 2021).

In summary, considering the zootechnical parameters and enzymatic assays of the processed brown seaweed biomass, the best performance was observed on the sample from the Mexican Caribbean coast using the lower level of inclusion (2.5 %) compared with the *Sargassum sp.* collected from the Gulf of California. The relevance of the present study is making a difference in the valorization of the massive invasion of *Sargassum* throughout the Caribbean. Furthermore, the recurrent blooms have derived more interest and research to collect the fresh algae and explore its potential in several fields such as bioenergy (Orozco--González et al., 2022), bioremediation (Saldarriaga-Hernandez et al., 2020), food packaging (Mohammed et al., 2020). More recently, in aquaculture, they reported positive results in enhancing growth and immune responses for several species of aquatic organisms (Abdelrhman et al., 2022), including shrimps (Abdel-Rahim et al., 2021a). Although the challenge did not finish with the use of Sargassum seaweed species, the new knowledge can address environmental issues more responsibly.

4. Conclusions

This study provides information on the potential use of the pelagic Sargassum spp. in aquaculture. In conclusion, adding 2.5 % of *Sargassum spp*. in diets for *L. vannamei* resulted in a significant improvement in growth performance, feed intake, and the highest activity of digestive enzymes compared with conventional formulations. These findings highlight an essential opportunity to encourage sustainable and profitable production practices within the aquaculture sector in which the circularity of nutrients on brown macroalgae enhances the future of food security. However, further research is needed on the characterization of the nutritional compounds from macroalgae and their specific effect on the activity of digestive enzymes and the immune system of *L. vannamei* and other organisms valued in aquaculture.

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CRediT authorship contribution statement

Conceptualization: E.M.M-M, R.P.S.,A.G.R., and A.P.R.; Methodology: Z.P.M, E.A.F-C, and D.R-G; Software: A.P.R., Z.P.M, and D.R-G; Formal analysis: Z.P.M, and A.P.R; Investigation: E.M.M-M, A.G.R., A. P.R, and Z.P.M; Resources: E.M.M-M, R.P.S, A.D.B. A.G.R and A.P.R. Data curation: A.P.R., Z.P.M, and D.R-G; Writing original draft: E.M.M-M., Z.P.M. E.A.F-C, A.G.R., A.P.R., R.G.A and D.R-G.; Writing- Review & Editing: E.M.M-M., J.E.S-H., H.M.N.I. A.D.B. and A.G.R.; Visualization: Z.P.M, E.A.F-C, and G.M.G.; Supervision: E.M.M-M., A.G.R, and A.P.R.; Project administration: E.M.M-M, R.P.S., and A.P.R.; Funding acquisition: E.M.M-M, R.P.S, A.D.B. A.G.R and A.P.R.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The data that has been used is confidential.

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References

Abbas, E.M., Al-Souti, A.S., Sharawy, Z.Z., El-Haroun, E., Ashour, M., 2023. Impact of dietary administration of seaweed polysaccharide on growth, microbial abundance, and growth and immune-related genes expression of the pacific whiteleg shrimp (*Litopenaeus vannamei*). Life 13 (2), 344. https://doi.org/10.3390/life13020344.

Abdel-Rahim, Mohamed, Bahattab, Omar, Nossir, Fatma, Al-Awthan, Yahya, Khalil, Riad H., Mohamed, Radi, 2021a. Dietary supplementation of brown seaweed and/or nucleotides improved shrimp performance, health status and cold-tolerant gene expression of Juvenile Whiteleg shrimp during the winter season. Mar. Drugs 19 (3). https://doi.org/10.3390/MD19030175.

Abdel-Rahim, Mohamed, Bahattab, Omar, Nossir, Fatma, Al-Awthan, Yahya, Khalil, Riad H., Mohamed, Radi, 2021b. Dietary supplementation of brown seaweed and/or nucleotides improved shrimp performance, health status and cold-tolerant gene expression of juvenile whiteleg shrimp during the winter season. Mar. Drugs 19 (3). https://doi.org/10.3390/MD19030175.

Abdelrhman, Abdelrhman M., Ashour, Mohamed, Al-Zahaby, Mohamed A., Sharawy, Zaki Z., Nazmi, Hany, Zaki, Mohamed A.A., Ahmed, Norhan H., Ahmed, Sherine R., El-Haroun, Ehab, Doan, Hien Van, Goda, Ashraf M.A., 2022. Effect of polysaccharides derived from brown macroalgae sargassum dentifolium on growth performance, serum biochemical, digestive histology and enzyme activity of hybrid red tilapia. Aquac. Rep. 25, 101212 https://doi.org/10.1016/J.AQREP.2022.101212.

Ahmad, Ashfaq, Hassan, Shadi W., Banat, Fawzi, 2022. An overview of microalgae biomass as a sustainable aquaculture feed ingredient: food security and circular economy. Bioengineered 13 (4), 9521. https://doi.org/10.1080/21655979.2022.2061148.

Amador-Castro, Fernando, García-Cayuela, Tomás, Alper, Hal S., Rodriguez-Martinez, Verónica, Carrillo-Nieves, Danay, 2021. Valorization of pelagic sargassum biomass into sustainable applications: current trends and challenges. J. Environ. Manag. 283. https://doi.org/10.1016/J.JENVMAN.2021.112013.

Amoriello, Tiziana, Mellara, Francesco, Amoriello, Monica, Ceccarelli, Danilo, Ciccoritti, Roberto, 2021. Powdered seaweeds as a valuable ingredient for functional breads. Eur. Food Res. Technol. 247 (10), 2431–2443. https://doi.org/10.1007/S00217-021-03804-Z.

Cai, J., Lovatelli, A., Aguilar-Manjarrez, J., Cornish, L., Dabbadie, L., Desrochers, A., Diffey, S., Garrido Gamarro, E., Geehan, J., Hurtado, A., Lucente, D., Mair, G., Miao, W., Potin, P., Przybyla, C., Reantaso, M., Roubach, R., Tauati, M., Yuan, X.,

2021. Seaweeds and Microalgae: an Overview for Unlocking Their Potential in Global Aquaculture Development. Circular No. 1229. FAO Fisheries and Aquaculture, Rome, FAO. https://doi.org/10.4060/cb5670en.

Castellanos-Ochoa, Carlos, Torres-Ochoa, Erika, Pacheco-Vega, Juan, Cortés-Sánchez, Alejandro, Espinosa-Chaurand, Daniel, Castellanos-Ochoa, Carlos, Torres-Ochoa, Erika, Pacheco-Vega, Juan, Cortés-Sánchez, Alejandro, Espinosa-Chaurand, Daniel, 2022. Crustacean chymotrypsin: estate of art. Abanico Vet. 12. https://doi.org/10.21929/ABAVET2022.9.

Cruz-SuÁrez, L.E., Tapia-Salazar, M., Nieto-LÓpez, M.G., Guajardo-Barbosa, C., Ricque-Marie, D., 2009. Comparison of Ulva Clathrata and the Kelps Macrocystis pyrifera and Ascophyllum nodosum as ingredients in shrimp feeds. Aquac. Nutr. 15 (4), 421–430. https://doi.org/10.1111/J.1365-2095.2008.00607.X.

Devault, Damien A., Modestin, Emma, Cottereau, Victoire, Vedie, Fabien, Stiger-Pouvreau, Valérie, Pierre, Ronan, Coynel, Alexandra, Dolique, Franck, 2021. The silent spring of sargassum. Environ. Sci. Pollut. Res. Int. 28 (13), 15580–15583. https://doi.org/10.1007/S11356-020-12216-7.

Edelson, Paul J., Robert, A. Duncan, 1981. Lowry and bradford assays for protein. Methods Stud. Mononucl. Phagocytes 337–343. https://doi.org/10.1016/B978-0-12-044220-1.50042-8.

Elizondo-González, Regina, Quiroz-Guzmán, Eduardo, Escobedo-Fregoso, Cristina, Magallón-Servín, Paola, Peña-Rodríguez, Alberto, 2018. Use of Seaweed Ulva lactuca for Water Bioremediation and as Feed Additive for White Shrimp Litopenaeus vannamei. PeerJ 2018 (3). https://doi.org/10.7717/PEERJ.4459/SUPP-3.

Galgani, F.G., Benyamin, Y., Ceccaldi, H.J., 1984. Identification of digestive proteinases of Penaeus Kerathurus (Forskål): a comparison with Penaeus Japonicus Bate. Comp. Biochem. Physiol. Part B: Comp. Biochem. 78 (2), 355–361. https://doi.org/10.1016/0305-0491(84)90043-9.

Gamboa-Delgado, Julián, Peña-Rodríguez, Alberto, Ricque-Marie, Denis, Cruz-Sáurez, Lucia Elizabeth, 2011. Assessment of nutrient allocation and metabolic turnover rate in pacific white shrimp Litopenaeus vannamei Co-Fed Live macroalgae ulva clathrata and inert feed: dual stable isotope analysis. J. Shellfish Res. 30 (3), 969–978. https://doi.org/10.2983/035.030.0340.

Ghosh, Alokesh Kumar, Panda, Sujogya Kumar, Luyten, Walter, 2021. Anti-vibrio and immune-enhancing activity of medicinal plants in shrimp: a comprehensive review. Fish. Shellfish Immunol. 117, 192–210. https://doi.org/10.1016/J.FSI.2021.08.006.

Gong, Jie, Xie, Yinjie, Yu, Kun, Yang, Ya'nan, Huang, Huiyang, Ye, Haihui, 2014. Characterization and expression analysis of chymotrypsin after bacterial challenge in the Mud Crab, scylla paramamosain. Genet. Mol. Biol. 37 (2), 381. https://doi.org/10.1590/S1415-47572014005000007.

Gunho, Eom, Jaebeom, Shin, Se-Jin, Lim, Kyu-Tae, Lee, Young-Seok, Han, Kyeong-Jun, Lee, 2020. Evaluation of dietary supplementation of Sargassum Horneri for Pacific White Shrimp Litopenaeus vannamei. Korean J. Fish. Aquat. Sci. 53 (6), 909–917. https://doi.org/10.5657/KFAS.2020.0909.

Hernández-Bolio, Gloria Ivonne, Fagundo-Mollineda, Adrián, Caamal-Fuentes, Edgar Emmanuel, Robledo, Daniel, Freile-Pelegrin, Yolanda, Hernández-Núñez, Emanuel, 2021. NMR metabolic profiling of Sargassum species under different stabilization/extraction processes. J. Phycol. 57 (2), 655–663. https://doi.org/10.1111/JPY.13117.

Immanuel, Grasian, Sivagnanavelmurugan, Madasamy, Balasubramanian, Varatharajan, Palavesam, Arunachalam, 2010. Effect of hot water extracts of brown seaweeds Sargassum Spp. on growth and resistance to white spot syndrome virus in shrimp penaeus monodon postlarvae. Aquac. Res. 41 (10), e545–e553. https://doi.org/10.1111/J.1365-2109.2010.02526.X.

Jahromi, Saied Tamadoni, Pourmozaffar, Sajjad, Jahanbakhshi, Abdolreza, Rameshi, Hossein, Gozari, Mohsen, Khodadadi, Mojgan, Sohrabipour, Jelveh, Behzadi, Siamak, Bazrkar, Noora, Nahavandi, Reza, Zahedi, Mohammad Reza, Moezzi, Maryam, 2021. Effect of different levels of dietary sargassum cristaefolium on growth performance, hematological parameters, histological structure of hepatopancreas and intestinal microbiota of Litopenaeus vannamei. Aquaculture 533, 736130. https://doi.org/10.1016/J.AQUACULTURE.2020.736130.

Kenney, Kevin L., Smith, William A., Gresham, Garold L., Westover, Tyler L., 2014. Understanding biomass feedstock variability. Biofuels 4 (1), 111–127. https://doi.org/10.4155/bfs.12.83.

Lapointe, Brian E., West, Lorin E., Sutton, Tracey T., Hu, Chuanmin, 2014. Ryther revisited: nutrient excretions by fishes enhance productivity of pelagic sargassum in the Western North Atlantic Ocean. J. Exp. Mar. Biol. Ecol. 458, 46–56. https://doi.org/10.1016/J.JEMBE.2014.05.002.

Lee, Po. Tsang, Tran, Hoang Trieu Quan, Huang, Huai Ting, Nan, Fan Hua, Lee, Meng Chou, 2020. Sargassum horneri extracts stimulate innate immunity, enhance growth performance, and upregulate immune genes in the white shrimp Litopenaeus vannamei. Fish. Shellfish Immunol. 102, 276–285. https://doi.org/10.1016/J.FSI.2020.04.049.

Louime, Clifford, Fortune, Jodany, Gervais, Gary, Rio, Rico, Juan, San, Rico, Puerto, 2017. *Sargassum* invasion of coastal environments: a growing concern. Am. J. Environ. Sci. 13 (1), 58–64. https://doi.org/10.3844/AJESSP.2017.58.64.

Mohammed, Akeem, Rivers, Arianne, Stuckey, David C., Ward, Keeran, 2020. Alginate extraction from sargassum seaweed in the Caribbean region: optimization using response surface methodology. Carbohydr. Polym. 245, 116419 https://doi.org/10.1016/J.CARBPOL.2020.116419.

Niu, Jin, Chen, Xu, Lu, Xue, Jiang, Shi Gui, Lin, Hei Zhao, Liu, Yong Jian, Huang, Zhong, Wang, Jun, Wang, Yun, Tian, Li. Xia, 2015. Effects of different levels of dietary Wakame (Undaria Pinnatifida) on growth, immunity and intestinal structure of juvenile Penaeus monodon. Aquaculture 435, 78–85. https://doi.org/10.1016/J.AQUACULTURE.2014.08.013.

Niu, Jin, Xie, Shi Wei, Fang, Hao Hang, Xie, Jia Jun, Guo, Tian Yu, Zhang, Yan Mei, Liu, Zhen Lu, Liao, Shi Yu, He, Ju. Yun, Tian, Li. Xia, Liu, Yong Jian, 2018. Dietary values of macroalgae porphyra Haitanensis in Litopenaeus vannamei under normal rearing and WSSV challenge conditions: effect on growth, immune response and intestinal microbiota. Fish. Shellfish Immunol. 81, 135–149. https://doi.org/10.1016/J.FSI.2018.06.010.

Olmos Soto, Jorge, 2021. Feed intake improvement, gut microbiota modulation and pathogens control by using bacillus species in shrimp aquaculture. World J. Microbiol. Biotechnol. 37 (2) https://doi.org/10.1007/S11274-020-02987-Z.

Olsson, Joakim, Gunilla, B.Toth, Albers, Eva, 2020. Biochemical composition of red, green and brown seaweeds on the Swedish west coast. J. Appl. Phycol. 32 (5), 3305–3317. https://doi.org/10.1007/S10811-020-02145-W/FIGURES/1.

Omont, Alexia, Quiroz-Guzman, Eduardo, Tovar-Ramirez, Dariel, Peña-Rodríguez, Alberto, 2019. Effect of diets supplemented with different seaweed extracts on growth performance and digestive enzyme activities of juvenile white shrimp Litopenaeus vannamei. J. Appl. Phycol. 31 (2), 1433–1442. https://doi.org/10.1007/S10811-018-1628-6.

Omont, Alexia, Elizondo-González, Regina, Escobedo-Fregoso, Cristina, Tovar-Ramírez, Dariel, Hinojosa-Baltazar, Patricia, Peña-Rodríguez, Alberto, 2021. Bacterial communities and digestive enzymatic activities of litopenaeus vannamei shrimp fed pre-digested seaweeds as a functional ingredient. J. Appl. Phycol. 33 (2), 1239–1251. https://doi.org/10.1007/S10811-021-02381-8/METRICS.

Orozco-González, Jorge Gabriel, Amador-Castro, Fernando, Gordillo-Sierra, Angela R., García-Cayuela, Tomás, Alper, Hal S., Carrillo-Nieves, Danay, 2022. Opportunities surrounding the use of sargassum biomass as precursor of biogas, bioethanol, and biodiesel production. Front. Mar. Sci. 8, 2066. https://doi.org/10.3389/FMARS.2021.791054/BIBTEX.

Oyesiku, O.O., Egunyomi, A., 2015. Identification and chemical studies of Pelagic Masses of *Sargassum natans* (Linnaeus) Gaillon and *S. Fluitans* (Borgessen) Borgesen (Brown Algae), found offshore in Ondo State, Nigeria. Afr. J. Biotechnol. 13 (10), 1188–1193. https://doi.org/10.4314/ajb.v13i10.

Panini, Roseane L., Pinto, Stephanie S., Nóbrega, Renata O., Vieira, Felipe N., Fracalossi, D.ébora M., Samuels, Richard I., Prudêncio, Elane S., Silva, Carlos P., Amboni, Renata D.M.C., 2017. Effects of dietary replacement of fishmeal by mealworm meal on muscle quality of farmed shrimp Litopenaeus vannamei. Food Res. Int. 102, 445–450. https://doi.org/10.1016/J.FOODRES.2017.09.017.

Plouguerné, Erwan, Cesconetto, Críscia, Cruz, Camila P., Pereira, Renato C., da Gama, Bernardo A.P., 2012. Within-thallus variation in polyphenolic content and antifouling activity in Sargassum vulgare. J. Appl. Phycol. 24 (6), 1629–1635. https://doi.org/10.1007/S10811-012-9826-0/FIGURES/4.

Praiboon, Jantana, Palakas, Somchit, Noiraksa, Tidarat, Miyashita, Kazuo, 2018. Seasonal variation in nutritional composition and anti-proliferative activity of brown seaweed, Sargassum oligocystum. J. Appl. Phycol. 30 (1), 101–111. https://doi.org/10.1007/S10811-017-1248-6/TABLES/8.

Ragaza, Janice Alano, Hossain, Md. Sakhawat, Koshio, Shunsuke, Ishikawa, Manabu, Yokoyama, Saichiro, Kotzamanis, Yannis, Brezas, Andreas, Kumar, Vikas, 2021. Brown seaweed (Sargassum Fulvellum) inclusion in diets with fishmeal partially replaced with soy protein concentrate for Japanese Flounder (Paralichthys olivaceus) juveniles. Aquac. Nutr. 27 (4), 1052–1064. https://doi.org/10.1111/ANU.13246.

Rioux, Laurie Eve, Turgeon, Sylvie L., 2015. Seaweed carbohydrates. Seaweed Sustain.: Food Non-Food Appl. 141–192. https://doi.org/10.1016/B978-0-12-418697-2.00007-6.

Rodríguez-González, Hervey, Orduña-Rojas, Javier, Villalobos-Medina, José P., García-Ulloa, Manuel, Polanco-Torres, Arturo, López-Álvarez, Ely S., Montoya-Mejía, Magnolia, Hernández-Llamas, Alfredo, 2014. Partial inclusion of Ulva lactuca and Gracilaria Parvispora meal in balanced diets for white leg shrimp (Litopenaeus vannamei). J. Appl. Phycol. 26 (6), 2453–2459. https://doi.org/10.1007/S10811-014-0272-Z/FIGURES/1.

Rushdi, Mohammed I., Abdel-Rahman, Iman A.M., Saber, Hani, Attia, Eman Zekry, Abdelraheem, Wedad M., Madkour, Hashem A., Hassan, Hossam M., Elmaidomy, Abeer H., Abdelmohsen, Usama Ramadan, 2020. Pharmacological and natural products diversity of the brown algae genus Sargassum. RSC Adv. 10 (42), 24951–24972. https://doi.org/10.1039/D0RA03576A.

Saldarriaga-Hernandez, Sara, Hernandez-Vargas, Gustavo, Iqbal, Hafiz M.N., Barceló, Damiá, Parra-Saldívar, Roberto, 2020. Bioremediation potential of Sargassum Sp. biomass to tackle pollution in coastal ecosystems: circular economy approach. Sci. Total Environ. 715, 136978 https://doi.org/10.1016/J.SCITOTENV.2020.136978.

Saldarriaga-Hernandez, Sara, Melchor-Martínez, Elda M., Carrillo-Nieves, Danay, Parra-Saldívar, Roberto, Iqbal, Hafiz M.N., 2021. Seasonal characterization and quantification of biomolecules from sargassum collected from Mexican Caribbean Coast – a preliminary study as a step forward to blue economy. J. Environ. Manag. 298, 113507 https://doi.org/10.1016/J.JENVMAN.2021.113507.

Saraswati, Puspo Edi Giriwono, Diah Iskandriati, Chin Ping Tan, Andarwulan, Nuri, 2020. In-vitro anti-inflammatory activity, free radical (DPPH) Scavenging, and Ferric Reducing Ability (FRAP) of Sargassum cristaefolium lipid-soluble fraction and putative identification of bioactive compounds using UHPLC-ESI-ORBITRAP-MS/MS. Food Res. Int. 137, 109702 https://doi.org/10.1016/J.FOODRES.2020.109702.

Schleder, Delano Dias, Peruch, Luiz Guilherme Buglione, Poli, Moisés Angel, Ferreira, Tamiris Henrique, Silva, Carlos Peres, Andreatta, Edemar Roberto, Hayashi, Leila, Vieira, Felipe do Nascimento, 2018. Effect of brown seaweeds on pacific white shrimp growth performance, gut morphology, digestive enzymes activity and resistance to white spot virus. Aquaculture 495, 359–365. https://doi.org/10.1016/J.AQUACULTURE.2018.06.020.

Serrano Jr., Augusto E., Santizo, Rena B., Tumbokon, Barry Leonard M., 2015. Potential use of the sea Lettuce Ulva lactuca replacing soybean meal in the diet of the black tiger shrimp Penaeus monodon Juvenile. Aquac. Aquar. Conserv. Legis. 8 (3), 245–252.

Sherwood, James, 2020. The significance of biomass in a circular economy. Bioresour. Technol. 300. https://doi.org/10.1016/J.BIORTECH.2020.122755.

Silva, Aurora, Rodrigues, Carla, Garcia-Oliveira, Paula, Lourenço-Lopes, Catarina, Silva, Sofia A., Garcia-Perez, Pascual, Carvalho, Ana P., Domingues, Valentina F., Barroso, M.F.átima, Delerue-Matos, Cristina, Simal-Gandara, Jesus, Prieto, Miguel A., 2021. Screening of bioactive properties in Brown Algae from the Northwest Iberian peninsula. Foods 10 (8). https://doi.org/10.3390/FOODS10081915.

Turner, R.Eugene, Rabalais, Nancy N., 1994. Coastal eutrophication near the Mississippi River delta, 1994 *368:6472* Nature 368 (6472), 619–621. https://doi.org/10.1038/368619a0.

White, M.J., Santhi, C., Kannan, N., Arnold, J.G., Harmel, D., Norfleet, L., Allen, P., DiLuzio, M., Wang, X., Atwood, J., Haney, E., Vaughn Johnson, M., 2014. Nutrient delivery from the Mississippi River to the Gulf of Mexico and effects of cropland conservation. J. Soil Water Conserv. 69 (1), 26–40. https://doi.org/10.2489/JSWC.69.1.26.

Yu, Ying Ying, Chen, Wen Dong, Liu, Yong Jian, Niu, Jin, Chen, Ming, Tian, Li. Xia, 2016. Effect of different dietary levels of Gracilaria lemaneiformis dry power on growth performance, hematological parameters and intestinal structure of Juvenile Pacific White Shrimp (Litopenaeus vannamei). Aquaculture 450, 356–362. https://doi.org/10.1016/J.AQUACULTURE.2015.07.037.

Yudiati, Ervia, Isnansetyo, Alim, Triyanto, Murwantoko, Handayani, Christina Retna, 2019. Alginate from Sargassum Siliquosum Simultaneously Stimulates Innate Immunity, upregulates immune genes, and enhances resistance of pacific white shrimp (Litopenaeus vannamei) against white spot syndrome virus (WSSV). Mar. Biotechnol. (N. Y., N. Y.) 21 (4), 503–514. https://doi.org/10.1007/S10126-019-09898-7.

Zhong, Biming, Robinson, Nicholas A., Warner, Robyn D., Barrow, Colin J., Dunshea, Frank R., Suleria, Hafiz A.R., 2020. LC-ESI-QTOF-MS/MS characterization of seaweed phenolics and their antioxidant potential. Mar. Drugs 18 (6). https://doi.org/10.3390/MD18060331.